CAT. No.       Pack Name       Packaging (Content)
BLT00032      HDL PREC 100     R1: 2 x 50 ml, R2 standard: 1 x 5 ml

**INTENDED USE**
Diagnostic reagent for quantitative in vitro determination of HDL Cholesterol in human serum and plasma.

**CLINICAL SIGNIFICANCE**
High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in liver as complexes of apolipoprotein and phospholipid and are capable of picking up cholesterol and carrying it from arteries to the liver, where the cholesterol is converted to bile acids and excreted into the intestine. An inverse relationship between HDL Cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized. Accurate measurement of HDL-C is of vital importance when assessing patient’s risk for CHD.

**PRINCIPLE**
Chylomicrons, LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatent and is estimated using ERBA Cholesterol reagent.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Phosphotungstic Acid</td>
</tr>
<tr>
<td></td>
<td>0.77 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Magnesium Chloride</td>
</tr>
<tr>
<td></td>
<td>17.46 mmol/L</td>
</tr>
<tr>
<td>R2</td>
<td>See bottle label</td>
</tr>
</tbody>
</table>

**STABILITY AND STORAGE**
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

**SPECIMEN COLLECTION AND HANDLING**
Use serum, plasma (EDTA). It is recommended to follow NCCLS procedures (or similar standardized conditions).

**CALIBRATION**
Calibration with the standard included in the kit.

**QUALITY CONTROL**
To ensure adequate quality control, it is recommended to use controls with assayed values.

**EXPECTED VALUES**

- Adults male: 35.3 - 79.5 mg/dl
- Adults female: 42.0 - 88.0 mg/dl

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

**WARNING AND PRECAUTIONS**
For in vitro diagnostic use. To be handled by entitled and professionally educated person. Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

**WASTE MANAGEMENT**
Please refer to local legal requirements.

**ASSAY PROCEDURE**

1. **PRECIPITATION**
Precipitation of LDL, VLDL and Chylomicrons.

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample or Calibrator</td>
<td>250 μl</td>
</tr>
<tr>
<td>Precipitating Reagent</td>
<td>500 μl</td>
</tr>
</tbody>
</table>

Mix well and allow the reaction mixture to stand for 10 minutes at room temperature, Centrifuge at 4000 r.p.m. (1800 x g) for 10 minutes to obtain a clear supernatent. Use the supernatent to determine the concentration of HDL cholesterol in the sample.

2. **CHOLESTEROL DETERMINATION**

- **Wavelength:** 500 (546) nm
- **Cuvette:** 1 cm

<table>
<thead>
<tr>
<th>Pipette into tubes marked</th>
<th>Blank</th>
<th>Calibrator</th>
<th>Test</th>
<th>Cholesterol Working Reagent</th>
<th>1000 μl</th>
<th>1000 μl</th>
<th>1000 μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>50 μl</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator</td>
<td>--</td>
<td>50 μl</td>
<td>--</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Supernatent</td>
<td>--</td>
<td>--</td>
<td>50 μl</td>
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</tbody>
</table>

Mix well, incubate for 10 min. at 37°C, or 12 min. at 30°C. Read the absorbance of the Calibrator and each test at 500 (546) nm or 505/670 nm for bichromatic analysers against reagent blank.

**CALCULATIONS**

- HDL Cholesterol = \( \frac{\text{Abs. of Test}}{\text{Abs. of Cal.}} \times \text{Concentration of Calibrator (mg/dl)} \)

Applications for automatic analysers are available on request.
REFERENCES